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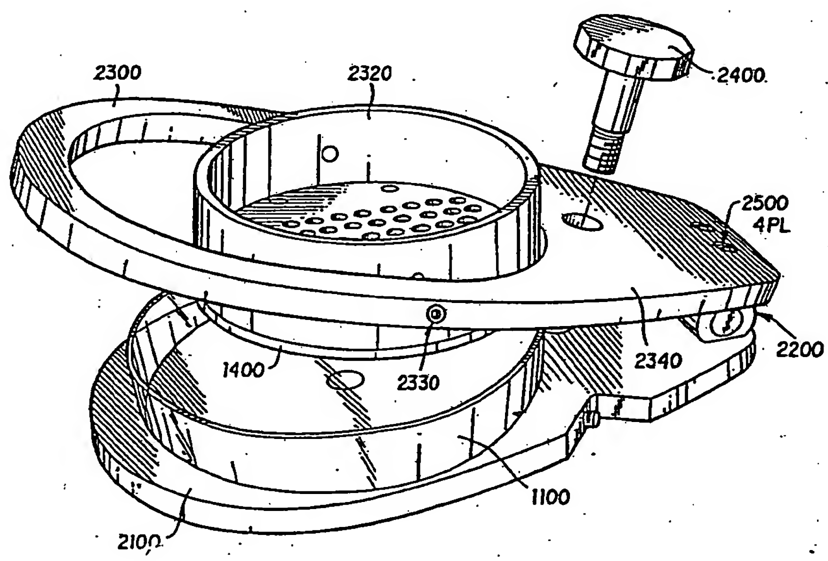
(54) Title: A REMOVABLE MULTI-WELL CHAMBER DEVICE AND METHOD FOR INCUBATION OF CULTURED CELLS WITH SMALL VOLUMES



(57) Abstract

A removable multi-well chamber device (2000) for incubation of cultured cells with small volumes having a first member (2320) with a planar surface and regularly spaced openings. A second member (1400) with first and second surfaces and having regularly spaced openings therethrough and is mountable to the first member (2320) so that the openings in the first (2320) and second (1400) members are in registration. The second member (1400) can be moved into and out of sealing contact with a culture surface (1100) so as to define multiple isolated wells for receiving samples. Two embodiments of the device (2000) are shown along with a method for detecting the presence of an antibody to a cell component disposed in one or more of the isolated wells.

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TITLE OF THE INVENTION

A REMOVABLE MULTI-WELL CHAMBER DEVICE AND METHOD FOR INCUBATION OF CULTURED CELLS WITH SMALL VOLUMES

1. Field of the Invention

The present invention relates generally to devices having multiple isolated wells for microscopy-based immunoassays and its use for detecting antibody binding to cultured cells. The present invention also relates to methods of using such multiple well devices for screening biological fluids such as hybridoma supernatants for the detection of cell-binding substances such as monoclonal antibodies.

2. Background

Immunofluorescence microscopy is a powerful technique for detecting the location of surface and intracellular antigens in individual cells. However, using standard methods, the processing of large numbers of samples for immunofluorescence is cumbersome and difficult.

Light microscopic immunocytochemistry has unique advantages for the detection of antibodies which bind to various cellular components. The ability to see a large number of cells simultaneously, to determine if an antibody reacts similarly with all cells in a sample and to discern to which

cellular components (e.g. organelles) the antibody binds, allows a large amount of information about antigen presence and localization to be acquired in a very short time using relatively few cells.

Different optical techniques can be used for light microscopic immunocytochemistry. Images may be detected by refraction, as in the detection of reaction product using immunoperoxidase methods. Objects may also be detected by their emission of light resulting in an image formed by refraction of the emitted light. Immunofluorescence is such a light emission method. Immunofluorescence detection is analogous to the detection of an object as a point source of light, such as in the visualization of individual stars in the night sky. Accordingly, objects as small as individual virus particles labeled with fluorescent dyes can easily be detected.

Immunofluorescence is an excellent screening method for selection of individual hybridoma clones when searching for monoclonal antibodies that react with particular components of cultured cells. Homogeneity of antigen distribution in a large cell population can be quickly assessed. The presence of antigen on substratum components, extracellular matrix elements, cell types of different shape, and on various organelles can be evaluated in a single image. This approach is especially useful for cell surface antigens which can be examined directly on living cells.

Unfortunately, current immunofluorescence processing methods are cumbersome and highly impractical for the primary screening of large numbers of small volume (<100 microliters) supernatant samples. This is specially true if short working distance microscope objectives of high resolution and sensitivity are required.

One known device used for preparing and testing large numbers of culture samples for testing is the microtiter tray,

which is well known in the art. Microtiter trays can have varying numbers of wells, typically 96 in an 8 x 12 array. A well is an individual chamber in which cells can be grown and an individual assay sample tested. The contents of each well are isolated from neighboring wells, allowing simultaneously testing of multiple samples (for example, 96 on one tray) with no cross-contamination. The lack of cross-examination is a major advantage of preparing multiple culture samples in a microtiter tray.

Microtiter trays, however, have significant disadvantages when microscopic examination of cells is desired. Specifically, the small diameter (typically about 6 mm) of each well does not permit insertion of the typical large diameter microscope objectives (approximately 30 mm) for close examination. Accordingly, images of high resolution and high sensitivity are difficult, if not impossible to achieve. In addition, cells must be planted with a sterile technique in each well individually; all washes and additions must involve transfer with each well, requiring the use of multiple pipette tips and large amounts of manual manipulation.

To avoid these limitations, cells are sometimes prepared on a flat slide or on a culture dish (typically 60, 100, or 150 mm in diameter). Flat microscope slides are known in the art. Slides have been modified by the use of a mask template to produce several (for example, 8) separate circular islands. A culture sample is placed on each island. In this configuration, large diameter microscope objectives can be brought to within short working distances, yielding images of high resolution and sensitivity. Such slides, however, also have distinct disadvantages. For example, preparing multiple culture samples on one flat slide increases the likelihood of cross-contamination. Each slide must be individually processed and includes a maximum of only 8 wells. Prior to incubation, cells or molecules from one sample may migrate to an

adjacent sample. In addition, for high resolution, immersion optics must be used and this requires the additional step of placing a thin, fragile coverglass over the slide. Furthermore, glass slides are a poorer substrate for attachment of cultured cells than plastic tissue culture plates.

Another conventional device that has been used to prepare culture samples for microscopic examination is a chamber slide. A typical chamber slide consists of 2 to 16 individual wells attached to a flat slide. The wells can be substantially removed from the flat slide. Breaking of the well, however, leaves a significant rim around the sample island. In addition, once the wells are broken off from the flat slide, they cannot be replaced thereon. Each well is isolated from the others.

A chamber slide has similar disadvantages to the microtiter tray in not allowing microscope objectives to approach the surface on which the cells adhere without removal of the wells. In addition, the more common chamber slides of 2-4 chambers are designed for relatively large volumes and small numbers of culture samples. Moreover, because breaking off the wells result in a rim surrounding each island a closely positioned microscope objective cannot be smoothly moved along the flat plate surface, which could severely limit the use of these chambers for automated examination using a motorized computer-controlled mechanical stage. Therefore, preparing a large number of small volume culture samples on a chamber slide is impractical. In addition, similar to the template slides described above, chamber slides require the addition of a coverslip for high resolution observation. Likewise, cells must be planted in each well separately using sterile technique. Moreover, prior to removing the wells, each wash procedure usually requires the pipetting of solution in each well.

Accordingly, one objective of the inventors was to

develop a device wherein a large number of culture samples could be rapidly prepared without cross-examination. Another objective of the inventors was to develop a device wherein a large number of culture samples could be rapidly tested using high powered microscopes with short working distance objectives. The inventors have achieved these objectives by developing an apparatus that, inter alia, combines the advantage of preparing large numbers of culture samples in a microtiter tray with the advantage of analyzing culture samples on a flat dish.

SUMMARY OF THE INVENTION

The present invention is a removable multi-well chamber device having a first member with a substantially rigid planar surface, with a plurality of regularly spaced openings therethrough. It also has a second member having first and second substantially parallel elastomeric surfaces. The second member also has a plurality of regularly spaced openings therethrough extending between its first and second parallel surfaces. The first surface of the second member is mountable to the planar surface of the first member, such that the openings in the first and second members are in registration. The present invention also has means for moving the second surface of the second member into and out of sealing contact with a culture surface, thereby defining multiple isolated wells for receiving test samples.

These multiple isolated wells can be easily removed after incubation. The device of the present invention allows the rapid and convenient processing of many test samples. Moreover, the device allows each culture sample to be examined using a short working distance, high numerical aperture oil or glycerol-immersion objective for maximum sensitivity and resolution. This device makes immunofluorescence a practical

method for the primary screening of hybridoma clones.

The present invention also includes a method for detecting the presence of antibodies to cell components using the device of the present invention. The method includes preparing a culture dish with a cell sample. The method also includes placing and securing the culture dish in the present invention, and then activating the means for moving the second surface of the second member into and out of sealing contact with the culture surface, thereby defining multiple isolated wells for receiving test samples. The method further includes steps for removing the multiple wells from the culture dish, and performing subsequent testing on an open culture dish.

BRIEF DESCRIPTION OF THE DRAWINGS

Various objects, features and attendant advantages of the present invention will be more carefully appreciated as the same becomes better understood from the following detailed description of the present invention. The detail description makes reference to the accompanying drawings, in which:

Figure 1 is an exploded view of the conceptual embodiment of the present invention, which shows the various major elements which make up the embodiment;

Figure 2(a) is a cross-sectional view of the conceptual embodiment of Figure 1, which includes a blown-up portion illustrating in enhanced detail the sealing contact area;

Figure 2(b) is a portion of the cross-sectional view of Figure 2(a) showing three well chambers with cultured cells and antibody solution therein;

Figure 3(a) is a top plan view of the upper plate assembled to the well plate assembly of the conceptual embodiment of Figure 1;

Figure 3(b) is a cross-sectional view of the upper plate assembled to the well plate assembly taken along section A-A

of Figure 3(a);

Figure 4(a) is a top plan view of the upper plate of the conceptual embodiment of Figure 1;

Figure 4(b) is a cross-sectional view of the upper plate taken along section B-B of Figure 4(a)

Figure 5(a) is a top plan view of the well plate assembly of the conceptual embodiment of Figure 1;

Figure 5(b) is a cross-sectional view of the well plate assembly taken along section C-C of Figure 5(b);

Figure 6(a) is a top plan view of the sealing member of the conceptual embodiment of Figure 1;

Figure 6(b) is a cross sectional view of the sealing member taken along section D-D of Figure 6(a);

Figure 7(a) is a perspective view of the lower plate assembly of the conceptual embodiment of Figure 1;

Figure 7(b) is a cross-sectional view of the lower plate assembly taken along section E-E of Figure 7(a);

Figure 8 is a flow chart indicating a method for screening surface antigens using the device of the present invention;

Figure 9 is a perspective view of the commercial embodiment of the present invention, which shows the various major elements of the embodiment;

Figure 10 is a cross-sectional view of a fully engaged commercial embodiment of the present invention;

Figure 11 is a plan view of the base plate assembly of the commercial embodiment of Figure 9;

Figure 12(a) is a partially cut-away perspective view of the pivoting bucket feature of the commercial embodiment of Figure 9;

Figure 12(b) is a partially cut-away perspective view of the sealing member assembled to the pivoting bucket;

Figure 13 is a top plan view of the top plate of the commercial embodiment of Figure 9; and

Figure 14 is a top view of the clamping screw of the commercial embodiment of Figure 9.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The conceptual embodiment 1000 and the commercial embodiment 2000 of the removable multi-well chamber device of the present invention will be described in reference to a method for preparing cells samples and analyzing multiple test samples of small volume. As will be appreciated by those skilled in the art of immunoassay, fluorescence microscopy, and cell culture, the removable multi-well chamber device of the present invention has many applications and provides advantages heretofore unavailable in convention laboratory devices.

Referring first to Figure 1, shown is an exploded view of conceptual embodiment 1000 of the present invention. The conceptual embodiment 1000 comprises a culture dish 1100, an upper plate 1200, a well plate assembly 1300, a sealing member 1400, a lower plate assembly 1500 and wing nuts 1600. A cross-sectional view of the conceptual embodiment 1000 is shown in Figure 2(a) and 2(b).

Figure 3(a) shows a top plan view of upper plate 1200 assembled to well plate assembly 1300. Figure 3(b) shows a cross-sectional view of Figure 3(a). As shown, upper plate 1200 and well plate assembly 1300 are generally secured by two screws 1270.

Figure 4(a) depicts a top plan view of upper plate 1200. As shown, upper plate 1200 is formed with a large circular opening 1220 and 2 counterbored thru-holes 1260. As can be seen from Figure 3(a) and 3(b), counterbored thru-holes 1260 and circular opening 1220 are provided so as to removably secure and provide access to well plate assembly 1300. Upper

plate 1200 also has 4 thru-holes 1240. As can best be seen from Figure 1, each thru-hole 1240 is adapted to receive a mounting post 1580 (to be described) therein. A wing nut 1600 is then fastened to each mounting post 1580 so that upper plate 1200 can be securely engaged with lower plate assembly 1500. In the conceptual embodiment, upper plate 1200 is made from plexiglass and formed by conventional machining processes. Upper plate 1200, however, can be made from a variety of materials and fabricated by a number of processes.

Figure 5(a) shows a top plan view of well plate assembly 1300. Well plate assembly 1300 comprises an upper well plate member 1320 and a lower well plate member 1340. Both upper well plate member 1320 and lower well plate member 1340 have a circular shaped exterior. Lower well plate member 1340 has a plurality of regularly spaced well openings 1342, which can, but is not limited to being, configured in an industry standard 6 x 10 array. Well openings 1342 are marked to indicate the row and column. Lower well plate 1340 is also formed to have an upper rigid planar surface 1344 and a lower rigid planar surface 1346. Lower well plate 1340 also has 4 threaded holes 1348 formed therein and positioned 90 degrees apart. In contrast, upper well plate 1320 is substantially recessed and has a top annular surface 1322 and a lower annular surface 1324. Upper well plate 1320 has 4 counter-bored thru-holes 1326 formed from top surface 1322. As shown in Figure 5(b), a screw 1360 is inserted into thru-holes 1326 and threaded into threaded holes 1348 so that lower well plate 1320 can be fastened to upper well plate 1340.

Upper well plate 1320 also has 2 threaded holes 1348. As best shown by Figures 3(a) and 3(b), a screw 1280 is inserted into thru-hole 1260 of upper plate 1200 and threaded into each threaded hole 1328 of upper well plate 1320. This allows upper plate 1200 to be securely fastened to well plate assembly 1300. As best shown by Figure 2(b), lower rigid

planar surface 1346 provides a mounting and sealing contact surface for sealing member 1400.

In the conceptual embodiment, well plate assembly 1300 is made from plexiglass and formed by conventional machining processes. Well plate assembly 1300, however, can be made from a variety of materials and fabricated by a number of processes.

Referring next to Figure 6(a), shown is a top plan view of sealing member 1400. As shown, sealing member 1400, can be, but is not limited to, a circular shape. Sealing member 1400 has a plurality of openings 1420 of the substantially the same diameter and configuration of well openings 1342 of well plate assembly 1300. As shown in Figure 6(b), sealing member 1400 is formed to have a first elastomeric surface 1440 and a second elastomeric surface 1460. As best shown by Figure 2(b), once engaged, elastomeric surfaces 1440 and 1460 are in sealing contact with lower rigid planar surface 1346 of well plate assembly 1300 and culture dish surface 1120 respectively. It is very important that the pattern of well openings 1342 of well plate assembly 1300 are in registration with openings 1420 of sealing member 1400. As shown in Figure 2(b), proper registration of openings 1342 and 1420 will create a cylindrical well 1140 on culture dish surface 1120.

In the conceptual embodiment 1000, sealing member 1400 is removably secured to lower rigid planar surface 1346 of well plate assembly 1300 by vacuum grease. Care must be taken not to get the grease into the well openings 1342. In the conceptual embodiment, sealing member 1400 is made from, but not limited to silicon rubber.

Figure 7(a) shows a perspective view of lower plate assembly 1500. Lower plate assembly 1500 comprises a lower plate 1520, a plurality of movable clamp assemblies 1540, a plurality of fixed clamp blocks 1560, 4 threaded mounting posts 1570, and a spacing member 1580. Figure 7(b) shows

cross-sectional view of lower plate assembly 1500.

Lower plate 1520 is formed with a recess 1522 adapted to receive plastic dish 1100. As shown in Figure 7(b), a spacing member 1580 is provided so that culture dish 110- will lie flat on recess are 1522. In the conceptual embodiment, a spacing member 1580 is made from, but not limited to, silicon rubber. Lower plate 1520 also has four thru-holes 1524. Thru-holes 1524 are each adapted to receive a mounting post 1570. Mounting post 1570 is secured to lower plate 1520 by nut 1585 fastened from the bottom side of lower plate 1520. Nuts 1585 also act as feet for conceptual embodiment 1000. Lower plate 1520 can be made from, but is not limited to, aluminum or stainless steel.

As shown in Figures 1 and 7(a), movable clamp assemblies 1540 are positioned approximately 90 degrees apart and opposite fixed clamp blocks 1560. Movable clamp assemblies 1540 are designed such as to force culture dish 1100 against fixed clamp blocks 1560. This configuration securely fixes culture dish 1100 in lower plate assembly 1500. Fixed clamp block 1560 is designed to smoothly fit the exterior of culture dish 1100. Although not shown, fixed block clamp 1560 may have a silicon rubber secured thereon so as to enhance contact with culture dish 1100. Fixed block clamp is secured to lower plate 1529 by conventional fastening configurations such as a threaded hole and screw. Movable clamp assembly 1540 comprises a fixed member 1542, a free member 1544 and a drive screw 1546. Fixed member 1544 is attached to lower plate 1520 by similar conventional configurations. In the conceptual embodiment, drive screw 1546 is threaded into fixed member 1542. When engaged, drive screw 1546 extends free member 1544 away from fixed member 1542 thereby exerting a force on culture dish 1100. Although not shown, free member 1544 may have a strip of silicon rubber applied thereon so as to enhance contact with culture dish 1100.

The following is a description of the general operation of the conceptional embodiment of the present invention.

In operation, culture dish 1100 with culture samples grown on culture surface 1120 is placed in recess 1522 of lower plate assembly 1520. Culture dish 1100 is then secured in position by tightening moveable clamp assemblies 1540. This action forces culture dish 1100 against fixed clamp blocks 1560. Sealing member 1400 is then secured to rigid planar surface 1346 of well plate assembly 1300. Well plate assembly 1300 is then secured to upper plate 1200 by screws 1270. Thereafter, the upper plate 1200/well plate assembly 1300/sealing member 1400 combination is engaged with lower plate assembly 1500 so that second elastomeric surface 1460 of sealing member 1400 is in sealing contact with culture dish surface 1120. Wing nuts 1600 are then fastened to threaded posts 1570 of lower assembly 1500 so as to provide secured engagement and contact pressure. Increased contact pressure assures that each well is isolated and that no gaps exist between wells. Furthermore, sealing member 1400, which is made from an elastomeric material, acts as a flexible sealing gasket. As such, any physical irregularities on culture dish surface 1100 or on rigid planar surface 1346 of lower well plate 1340 will not affect isolation between the wells. Thus, when assembled, the device of the present invention creates 64 separate wells on culture dish surface 1120 in a configuration identical to a portion of a standard 96-well plate (9 mm between centers). Following the necessary incubation step or steps, the device can then be disengaged. Culture dish 1100 can then be removed. Culture dish 1100 is now an "open dish" with individual islands of cells not obstructed by well chambers or any other material. The position of the islands of cells corresponding to each well is established using a template (not shown). The template is fastened to the outside of the bottom of the culture dish 1100 and is configured in

the same pattern as the chamber wells.

The major advantage of the above inventive concept is that a culture dish can be quickly disassembled leaving islands of cells directly accessible. This allows examination from the top of the dish with short working distance microscope objectives. Unlike 96-well microtiter trays, the optical path to the cells is not restricted and large diameter objective lenses can be used with an upright microscope. In addition, a closely positioned microscope objective can be smoothly moved along a culture dish during scanning.

The following is the a method of using the removable multi-well device in the context of screening hybridomas growing in 96 well microtiter trays for antibodies that bind to surface antigens of living cells. A flow-chart summary of the protocol sequence is shown in Figure 8.

Step 1: Cells are plated in 150 mm dishes the day before they are used for assay. Some cell types are relatively poorly adherent to the plastic surface, and the addition of solutions to the small wells in the assembled chamber creates strong currents which can dislodge poorly adhering cells. For this reason, the inventors routinely use dishes that have been coated with Cell-Tak™ (Biopolymers; Collaborative Research) prior to plating the cells. Cell-Tak™ is applied to the area of the 150 mm dish that will form the bottom of the wells when the chamber is finally assembled. The cells are then plated in conventional culture medium, and assays are performed within 1-3 days.

For screening, one 150 mm culture dish is used with one chamber device creating 64 wells per dish. Thus, for a hybridoma fusion in which fused cells have been plated in 600 microtiter tray wells, 10 culture dish are used. With some practice, 5-6 chambers can be handled at one time by one person, requiring about three hours for processing. Therefore, a 600-well fusion can be screened in one day, half in

the morning and half in the afternoon.

The chamber device of this invention can be used for incubations with living cells, or can be used after cells are fixed and permeabilized. Although designed to be used with adherent cells, the device can also be used with cells in suspension by causing their attachment to the dish using Cell-Tak™ or poly-L-lysine, both methods of which are known in the art.

Step 2: The cells are washed free of their medium and cooled to about 4°C (to prevent endocytosis of bound antibodies) by addition of chilled phosphate-buffered saline with Ca^{++} and Mg^{++}, containing 2 mg/ml crystalline bovine serum albumin (Miles) (PBS-BSA).

Step 3: The removable multi-well chamber device is assembled with culture dish 1100. The lower plate 1520 is kept resting on wet ice. The cells are never allowed to dry during all the manipulations.

Step 4: Culture supernatants being tested are added to the wells of the device. The PBS-BSA in each row of wells is removed by a multiple channel suction manifold (Inticorp) leaving a minimal volume of PBS-BSA at the bottom to keep the cells from drying out. Then 100-200 microliters of supernatants are transferred from the 96-well microtiter tray in which the hybridomas are cultured using a multiple channel micropipettor (Flow Labs). This operation is performed row by row (or column by column), so that there is no risk of wells drying out. The cells are allowed to incubate for about 60 minutes with the hybridoma supernatants at about 4°C.

Step 5: After the incubation with the supernatants, the individual fluids are removed and the wells washed to prevent mixing of supernatants between wells. This can be accomplished in the same way as the addition of supernatants in the previous step using a suction multiple channel suction manifold, and a micropipettor for the addition of wash buffer.

If one wishes to save the supernatants, they would be removed by micropipettor and replaced with PBS-BSA. After removal of test supernatants (or prior to this step if the supernatants are not to be saved), the wells can be washed simply by inverting the chamber. Any solution adhering to the surface of the top of the chamber falls out when the chamber is inverted, but the solutions in the wells do not because of the small size of the wells and the surface tension of the aqueous solutions. However, if the chamber is vigorously shaken once, the fluid contents of each well spills out, without mixing with adjacent wells. It is then important to add rapidly wash solution to the wells so that they will not dry. Once the chamber is turned upright, it can be "flooded" with PBS-BSA over its top, shaken gently, and the wells will simultaneously fill with buffer. The inversion and shaking can then be repeated, and the washing is completed in one or two minutes by repeating the pouring, inversion and shaking sequence.

<u>Step 6</u>: The removable multi-well chamber device is then disassembled, taking care to avoid drying of the cells. The culture dish 100 is removed.

<u>Step 7</u>: The "second step" reagent is now added to the dish in "bulk" as a large volume incubation. For example, in screening mouse hybridomas, we use affinity-purified goat anti-mouse IgG (H+L) conjugated to rhodamine (Jackson ImmunoResearch) at 50 ug/ml in PBS-BSA. After a 30-min incubation at about 4°C, the dishes are washed with cold PBS-BSA, then with PBS at room temperature and the rhodamine labeled cells are then fixed in 3.7% formaldehyde in PBS for 15 min at room temperature. This preserves the cells and fixes the antibody label in place.

<u>Step 8</u>: The culture dish containing the stained fixed cells is washed again with PBS.

<u>Step 9</u>: The culture dish is then filled with glycerol to create a 1-2 mm thick layer. Once the cells are fixed and

immersed in glycerol, the dishes can now be examined microscopically or stored at 4°C in the dark for weeks prior to microscopic examination.

Step 10: A circulate template made from thin celluloid punched in the pattern of the wells, and labeled with the numbers of the rows, is attached to the bottom of the outside of the disk using double-sticky tape. A tape tab on one side allows the template to be easily removed and transferred to other plates for viewing on the microscope. Orientation of the template is accomplished by marking a single line on the outside of the dish at the top of the pattern when it is assembled in the chamber, then aligning this mark in position with the template when it is attached with double-sticky tape. The sample template can be used over and over. Of course, each chamber is completely reusable.

Step 11: The culture dish containing fixed, stained cells is viewed under a fluorescence microscope. The microscope we have used for viewing is a Zeiss standard research microscope equipped with rhodamine epifluorescence optics, a substage phase contrast condenser, and a larger-than-normal flat stage plate made from one piece of flat 3/16 inch thick aluminum plate, 7-1/2" square, with a 1" diameter hole in its center. This plate is mounted by two screws in the original carrier for other mechanical stage holders. This stage allows the entire dish to be viewed by moving from "island to island" (that is, the former location of the removable wells) guided by the template pattern. The position of the center of the field can be observed by looking for the refraction of the light from the bottom of the plate from the phase contrast light source when the objective is moved to the side. The mechanical stage controls still function to allow fine movement of the field within each well. The objectives typically used are Zeiss Planapochromats, 40x, N.A. 1.0 or 63x, N.A. 1.4 oil immersion lenses. While these objectives

are designed for use with oil immersion, they provide a very clear high resolution image when used with glycerol immersion. Other objectives are available (for example, lenses with correction for glycerol immersion, although their numerical apertures are slightly lower.

The dish is placed on the stage plate and the objective is immersed in the glycerol solution. Cells are viewed using phase contrast substage illumination. The fluorescence image can then be seen by simply blocking the phase contrast light path from below the stage.

With practice, a 60-well dish can be examined in 15-20 minutes. Each dish has four additional wells for appropriate positive and negative control treatments, which should be examined first before viewing the rest of the plate. The images detected using this method are of high resolution and brightness.

An alternative approach to manual examination that is allowed by this device is the use of an automated computer-controlled mechanical stage. The automated computer-controlled mechanical stage could be used with or without a computer detection system for positive and negative fluorescence signals.

OTHER EMBODIMENTS AND APPLICATIONS

1. Other Embodiments:

The commercial embodiment 2000 of the present invention will now be described.

Referring next to Figure 9, a perspective view of the commercial embodiment 2000 is shown. Commercial embodiment 2000 generally comprises a culture dish 1100 fastened to a base plate assembly 2100. Base plate assembly 2100 is rotatably attached to a top plate assembly 2300 by a hinge 2200. Once engaged, base plate assembly 2100 and top plate

assembly 2300 are secured by a clamping screw 2400.

Culture dish 1100 of the commercial embodiment is a standard 150 mm diameter disposable plastic petri dish. Although not clearly shown, standard culture dishes 1100 have a flanged lip which protrudes outward along its bottom outside surface. The effects of this flanged lip, as will be discussed, is accounted for by the present invention. As will be obvious to one skilled in the art, many other culture dishes could be substituted for the petri dish of the present invention.

Base plate assembly 2100 is further described in Figure 11. Base plate assembly 2100 comprises generally a base plate 2120, a plurality of fixed pins 2140, and a movable pin 2160.

Base plate 2120 comprises a recessed area 2122. Recessed area 2122 provides a mounting area for culture dish 1100. Recessed area 2122 should be relatively flat and have a clear gloss or other mirror like surface. A clear gloss makes it easier to view test samples placed in the wells overlying culture dish 1100. Also provided is a undercut area 2123. As discussed, standard culture dishes 1100 have a flanged lip which protrudes outward along its bottom outside edge. Undercut area 2123 is provided so that culture dish 1100 will lie flat on recessed area 2122. Base plate 2120 also comprises thru-holes 2124. As can be seen from Figure 9, thru holes 2124 receive screws 2500 which correspondingly secure hinge 2200 to base plate assembly 2100. Base plate 2120 further comprises a threaded hole 2126 for receiving clamping screw 2400. Although not shown, base plate 2120 may also comprise mounting feet attached to its underside surface.

Although in the commercial embodiment of the present invention, base plate 2120 comprises thru holes 2124 and thread hole 2126, it should be readily apparent that other fastening hole designs could be easily employed. Likewise,

base plate 2120, although made from aluminum in the commercial embodiment, could be made from a variety of materials. Although thermally conductive materials are preferred, they are not essential.

As discussed above, base plate assembly 2200 further comprises fixed pins 2140, and a movable pin 2160. Fixed pins 2140 and movable pin 2160 act in combination to securely fasten culture dish 1100 to recess area 2122. Fixed pins 2140 and movable pin 2160 are spaced about 120 degrees from each other and located on the side wall of recess area 2122. Movable pin 2160 is screwably fastened to the side wall of recess area 2122. The rotation of movable pin 2160 causes culture dish 1100 to be securely forced against fixed pins 2140.

It should be obvious to one skilled in the art, however, that many other means exist for securely fastening culture dish 1100 to base plate 2120. Such other means may include adhesive mounting or magnetic coupling.

As can be seen from Figure 9, top plate assembly 2300 generally comprises a pivoting bucket 2320 rotatably attached to a top plate 2340 and sealing member 1400. Sealing member 1400 is substantially the same as that used in the conceptual embodiment 1000.

Figure 12 shows a partial cut-away view of pivoting bucket 2320. Pivoting bucket 2320 is generally a cylinder closed at one end. The closed ends acts as a floor 2322. In the commercial embodiment, floor 2322 has a thickness of approximately 0.50 inches. Floor 2332 has a plurality of regularly spaced well openings 2324 provided therein. In the commercial embodiment, well openings 2324 are approximately 6 mm in diameter and regularly spaced in a 10 x 6 matrix pattern. The size, configuration and number of openings 2324 are dependent, inter alia, on the size of culture dish 1100 used and the number and volume of culture samples to be

tested. Pivoting bucket 2320 also comprises a relatively flat and rigid bottom planar surface 2326.

Pivoting bucket 2320 may further comprise 4 additional openings 2327 (not shown) centered over the top row. Additional openings 2327 provide wells for use as positive and negative controls in the assays. Additional openings 2327 could also be used for 4 additional sample wells.

Pivoting bucket 2320 also comprises mounting lugs 2328 fastened to bottom planar surface 2326. As shown in Figure 12(b), lugs 2328 are adapted to securely fasten with counterbored thru-holes 1480 of sealing member 1400. In the commercial embodiment, 5 lugs 2328 are employed and spaced in a unique pattern on rigid planar surface 2326. This unique pattern ensures that openings 2324 are always properly registered with openings 1420 of sealing member 1400.

Pivoting bucket 2320 also comprises two threaded side holes 2329. As shown in Figure 9, threaded holes 2329 receive a mounting screw 2330 such as to rotatably fasten pivoting bucket 2320 to top plate 2340.

Sealing member 1400 is substantially the same configuration for both the commercial embodiment and the conceptual embodiment (previously discussed). Figures 6(a) and 6(b) show sealing member 1400 in detail. The only structural difference is that for the commercial embodiment, counterbored holes 1480 are provided. Counterbored holes 1480 are adapted to receive and secure lug 2328 of well bucket 2322.

Once the device of the present invention is engaged, elastomeric surfaces 1440 and 1460 are in sealing contact with rigid planar surface 2326 of pivoting bucket 2320 and culture dish 1120 respectively. It is very important that the pattern of openings 2324 of pivoting bucket 2320 are in registration with the openings 1420 of sealing member 1400. As shown in Figure 10, proper registration of openings 2324 and 1420 will create a cylindrical well 1140. Cylindrical well 1140 will

have a length equal to the combined thicknesses of floor 2322 and sealing member 1400. Accordingly, 64 separate cylindrical wells 1140 have been formed on culture dish surface 1120. Sealing contact provides isolation between wells so that no cross-contamination will occur. In addition, changing the thickness of floor 2322 or of sealing member 1400 will change the capacity of each well 1140 accordingly.

In the commercial embodiment, sealing member 1400 is preferably made from a lower durometer elastomeric material. Having elastomeric properties ensures a tight seal which completely isolates each well 1140. The elastomeric material conforms to any irregularities present on the rigid culture dish surface 1120 or on rigid planar surface 2326 of pivoting bucket 2320.

Top plate 2340 is further described in Figure 13. As shown, top plate 2340 comprises an opening 2342. The size of opening 2342 corresponds to the size of pivoting bucket 2320 employed. For example, a larger pivoting bucket 2320 would require a larger opening 2342. It can also be seen that opening 2342 is larger than pivoting bucket 2320. The increased opening size 2342 creates a handle 2343 which can be used for transporting removable multi-well chamber device of the present invention.

Top plate 2340 further comprises threaded holes 2348. As shown in Figure 9, threaded holes 2348 receive mounting screws 2330 such as to rotatably fasten pivoting bucket 2320 to top plate 2340. It is important that the threaded holes 2348 be positioned properly such that pivoting bucket 2320 can rotate. The ability of pivoting bucket 2320 to rotate is important so that when the top plate assembly 2300 is lowered, rigid planar surface 2326 of pivotal bucket 2320 will always remain approximately parallel to culture dish surface 1120. This will prevent rigid planar surface 2326 of pivoting bucket 2320 from scraping culture samples that may already exist on

culture dish surface 1120 prior to full engagement of the present invention.

Top plate 2340 also comprises thru-holes 2344. As can be seen from Figure 9, thru-holes 2344 receive hinge screws 2500 that fasten top plate 2340 to hinge 2200. Top plate 2340 further comprises a thru-hole 2346 for receiving clamping screw 2400.

Top plate 2120 of the commercial embodiment is made from aluminum and shaped in the above described configuration. Top plate 2120, however, can be made from a variety of materials and shaped in numerous configurations. Top plate 2120 of the commercial embodiment is formed by conventional machining processes. Top plate 2120, however, can be formed from a variety of processes such as casting.

Figure 14 is a detail view of clamping screw 2400. Although not present in the commercial embodiment, clamping screw 2400 may incorporate a torque overload member. A torque overload member would be adapted so as to prevent the threads of clamping screw 2400 from stripping in response to an excessive torque. Clamp screw 2400 is formed to have a handle portion 2420 so that quick fastening of the commercial embodiment 2000 can be made. Clamp screw 2400 can be made from conventional materials such as stainless steel and formed by conventional machining processes.

The following is a general description of the operation of the commercial embodiment of the present invention.

A culture dish 1100 is securely fastened to base plate 2120 by tightening movable pin 2160. Top plate assembly 2300 is then engaged (lowered). When assembled, the device of the present invention creates 64 separate wells on culture dish surface 1100 in a configuration identical to a portion of a standard 96-well plate (9 mm between centers). To ensure that contents of the wells remain isolated, top plate assembly 2300 is pressure-secured to base plate assembly 2100 via clamping

screw 2400. This ensures that no gaps are present between wells. Following the necessary incubation step or steps, the device can then be disengaged. Culture dish 1100 can then be removed. As in the conceptual embodiment, culture dish 1100 is now an "open dish" with individual islands of cells not obstructed by well chambers or any other material. The position of the islands of cells corresponding to each well is established using a template (not shown). A template (not shown) is fastened to the outside of the bottom of the culture dish 1100 and is marked in the same pattern as the chamber wells openings 2324 of well bucket 2320.

2. Other Applications:

The removable multi-well chamber device can also be used to examine the location of intracellular antigens. For this purpose, cells must be fixed and permeabilized prior to incubation with the primary antibody. For example, cells have first been fixed in 3.7% formaldehyde in PBS (room temperature, 15 min) followed by antibody incubations in the continuous presence of 0.1% saponin (Sigma) for permeabilization. The resolution and sensitivity of these methods is comparable to that seen with other immunofluorescence processing procedures.

In addition to examining binding of different antibodies to a single cell type, the removable multi-well chamber device can be used to plate different cell types onto the culture dish after the chamber is assembled. Different antibodies are then added to the wells individually, or if a single antibody is to be tested with multiple cell types on one dish, the chamber can be disassembled after plating the cells and the antibody added to the dish in "bulk." Since these assays generally do not require long periods of cell growth, sterilization of the parts of the device which come in contact with the cells using ethanol is sufficient. Depending on the materials used to construct the device, different methods of

sterilization could be used, as is well-known in the art.

The removable multi-well chamber device of this invention is useful in a multitude of assays in addition to those described above. For example, virus infectivity, or DNA transfectivity, is tested by adding virus containing fluids or DNA precipitates to the wells of the chamber placed on a culture dish containing a particular cell type. Efficiency of infection (or transfection) is assayed using a fluorescence assay for the production of an appropriate marker protein. Alternatively, in situ hybridization of a visually labeled nucleic acid probe can be performed.

Still another use of the removable multi-well chamber device is the examination of bacterial or parasite suspensions for the presence of particular antigens by attaching the organisms to the dish by similar methods to those described for animal cells in suspension. The removable multi-well chamber device can also be used to incubate different cell populations in small volumes by assembling the device onto the culture dish prior to plating the cells. This is an advantage in examining samples in which only small numbers of cells are available, such as clinical samples from effusions or peripheral blood, or for individual clones of cultured cells that can be harvested from other culture dishes.

The device of the present invention can also be used to allow incubations of small volumes over larger objects, such as tissue sections or pieces of filter paper that are smaller than 6 mm in diameter and are attached to the dish surface. Alternatively, other well patterns on larger pieces could be used, in which sections or objects larger than 6 mm could be incubated in larger wells.

Additionally, because the device of the present invention allows the reassembly of wells at a later time in the same register as the original well pattern, serial incubations that alternate between common versus isolated well steps in a

procedure can be performed. Also possible would be the inclusions of patterns that enclosed a subset of the wells in a subsequent step; for example, of 60 original wells incubated, a later step could have a well pattern that enclosed 10 wells at a time, allowing 6 different subsequent incubation conditions on subsets of the original wells.

There are many other applications for this type of chamber, a device which transiently creates individual small wells in a large culture dish, and then can be disassembled from the dish at a later time. The foregoing description is intended primarily for purposes of illustration. This invention may be embodied in other forms or carried out in other ways without departing from the spirit or scope of the invention. Modifications and variations still falling within the spirit or the scope of the invention will be readily apparent to those of skill in the art.

WHAT IS CLAIMED IS:

1. A removable multi-well chamber device comprising:

a first member having a substantially rigid planar surface with a plurality of regularly spaced openings therethrough;

a second member having first and second substantially parallel elastomeric surfaces, said second member having a plurality of regularly spaced openings therethrough extending between said first and second parallel surfaces, the first surface of said second member being mountable to said planar surface of said first member, such that the openings in said first member are in registration with the openings in said second member; and

mean for moving the second surface of said second member into and out of sealing contact with a culture surface to thereby define multiple isolated wells for receiving samples.

2. A removable multi-well chamber device according to claim 1, wherein said first member comprises a well member.

3. A removable multi-well chamber device according to claim 2, wherein said moving means comprises an upper plate and a bottom plate.

4. A removable multi-well chamber device according to claim 3, wherein said moving means further comprise a means for securing a culture dish to said bottom plate.

5. A removable multi-well chamber device according to claim 4, wherein said securing means comprises a fixed pin and a movable pin.

6. A removable multi-well chamber device according to claim 5, wherein said plurality of openings are circular.

7. A removable multi-well chamber system comprising:

a plurality of multi-well chamber devices, wherein each multi-well chamber device comprises:

a first member having a substantially rigid planar surface with a plurality of regularly spaced openings therethrough;

a second member having first and second elastomeric surfaces, said second member having a plurality of regularly spaced openings therethrough extending between said first and second parallel surfaces, the first surface of said second member being mountable to said planar surface of said first member, such that the openings in said first member are in registration with the openings in said second member; and means for moving the second surface of said second member into and out of sealing contact with a culture surface to thereby define multiple isolated wells for receiving samples.

8. A method for detecting the presence of an antibody to a cell component in a fluid comprising the steps of:

(a) adhering said cell to a culture dish;
(b) placing and securing the culture dish in the multi-well chamber;
(c) engaging the multi-well chamber device so that the second surface of the second member is in contact with the culture dish surface to thereby create a plurality of wells therein;
(d) introducing said fluid to said well of said device;
(e) removing said device from said culture dish;
(f) incubating in said dish a labelled reagent capable of binding to said antibody; and

(g) detecting said antibody by examining said cells with a microscope to detect said labelled reagent.

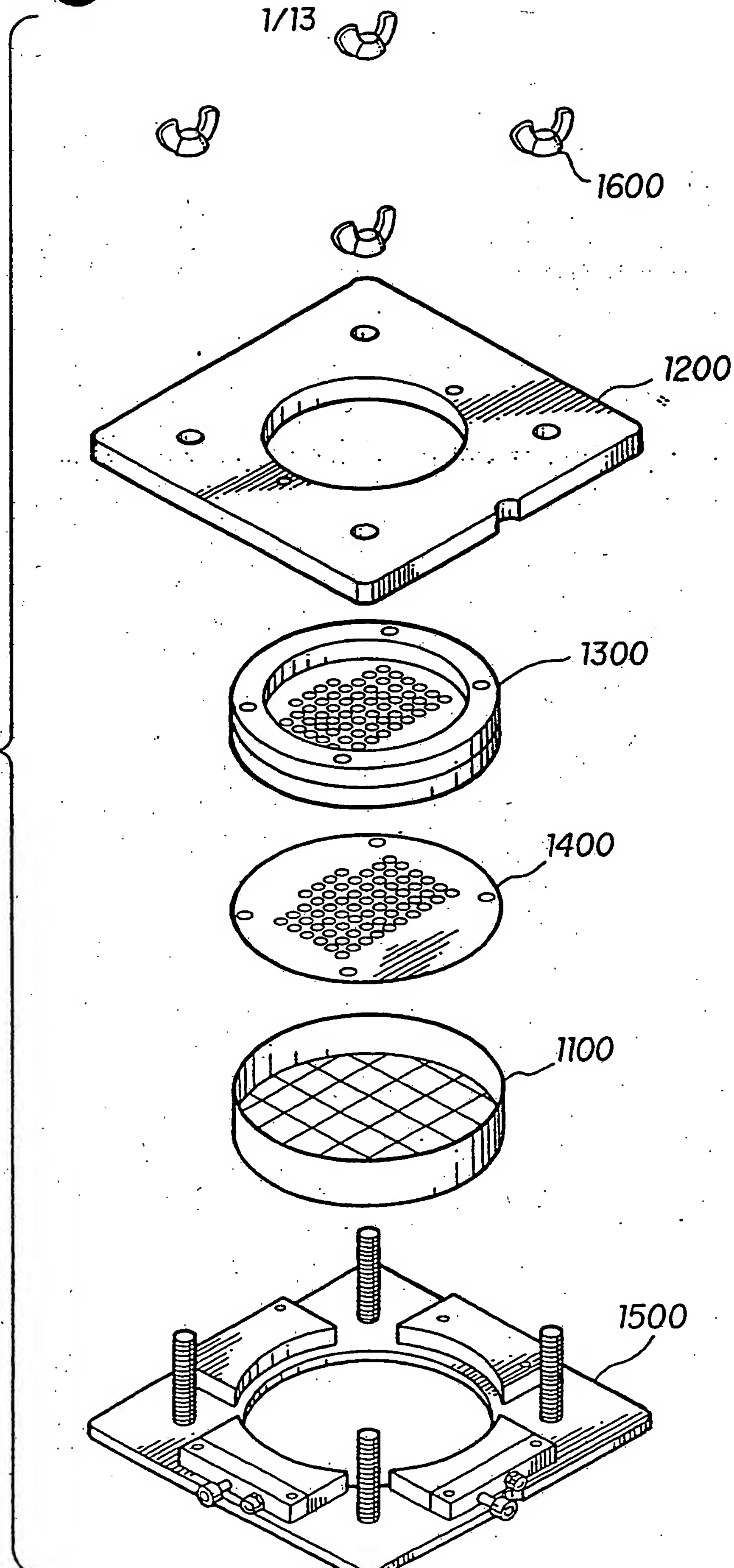

FIG. 1

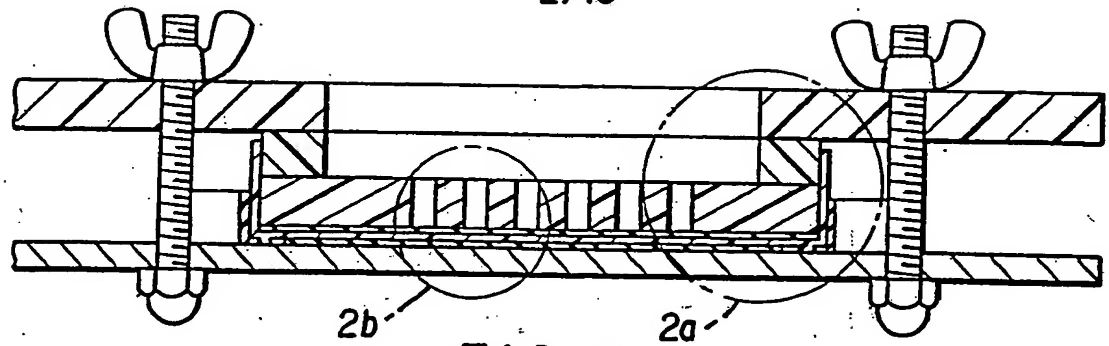

FIG. 2

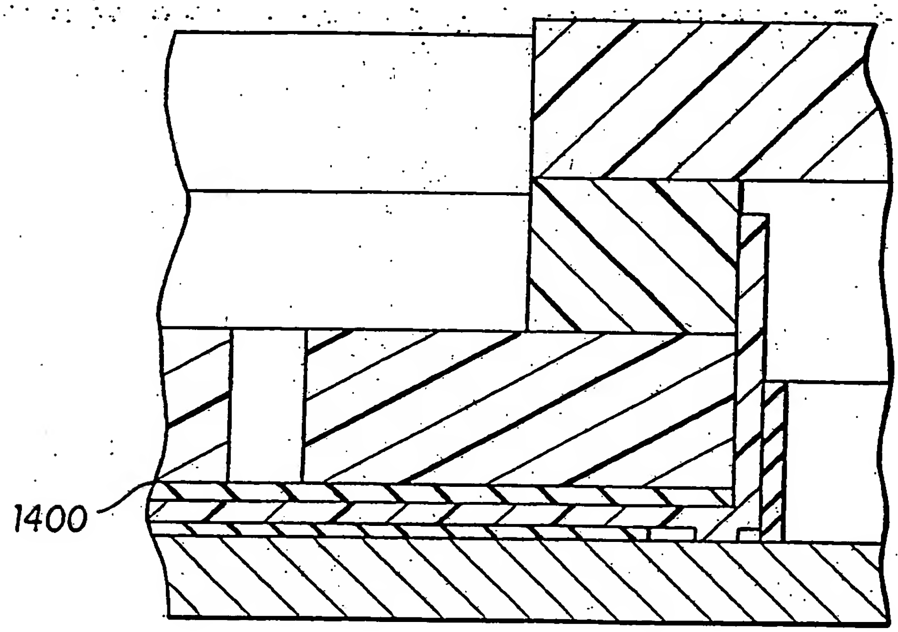

FIG. 2a

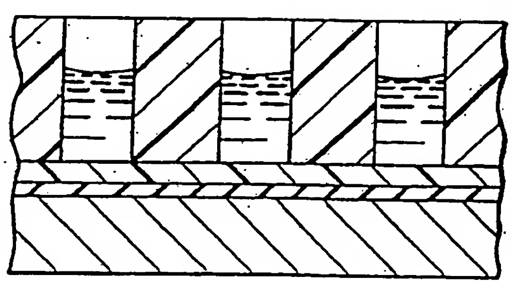

FIG. 2b

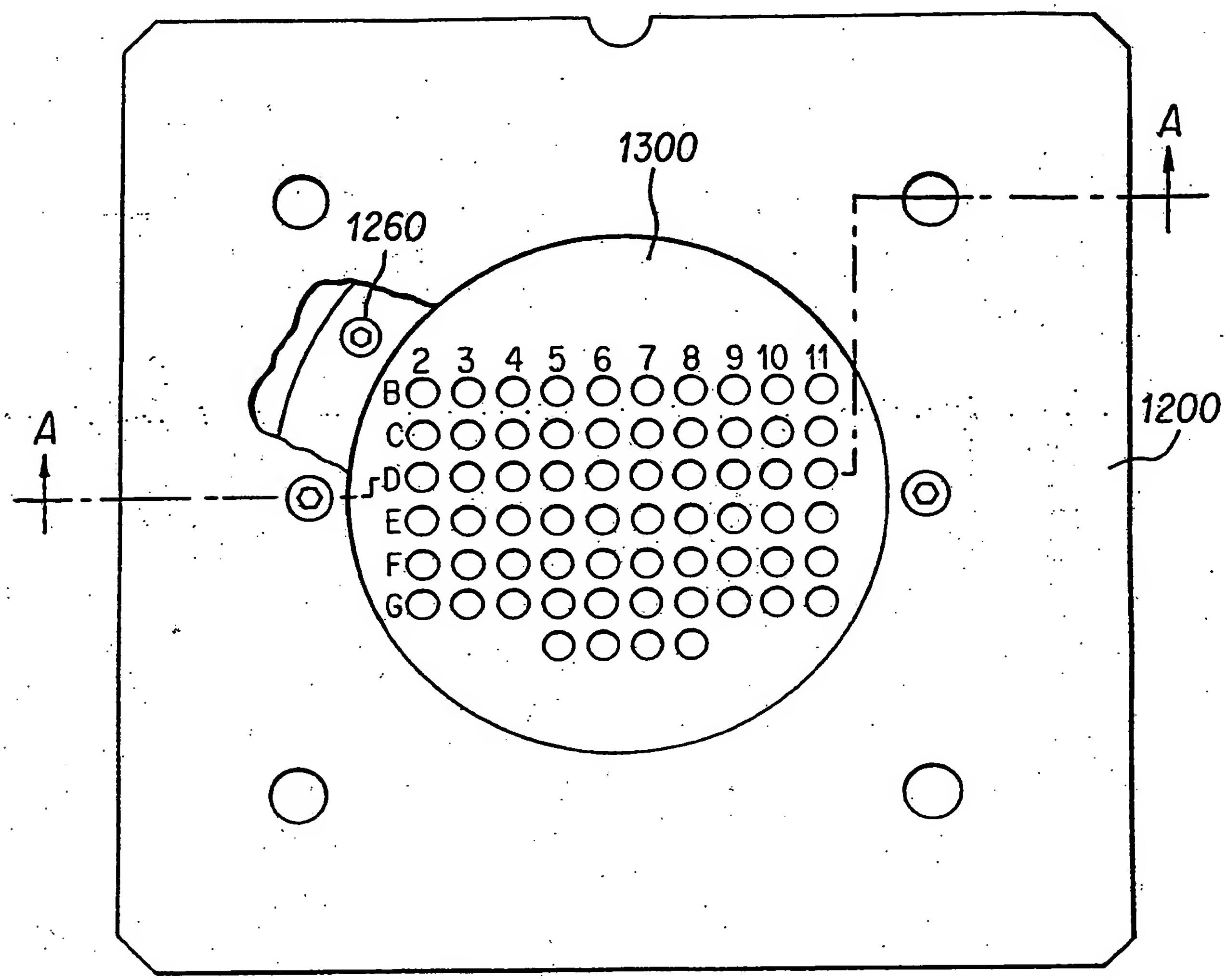

FIG. 3a

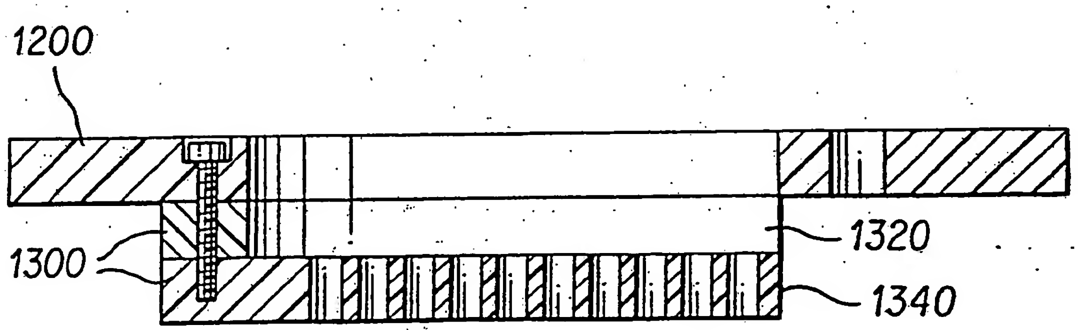

FIG. 3b

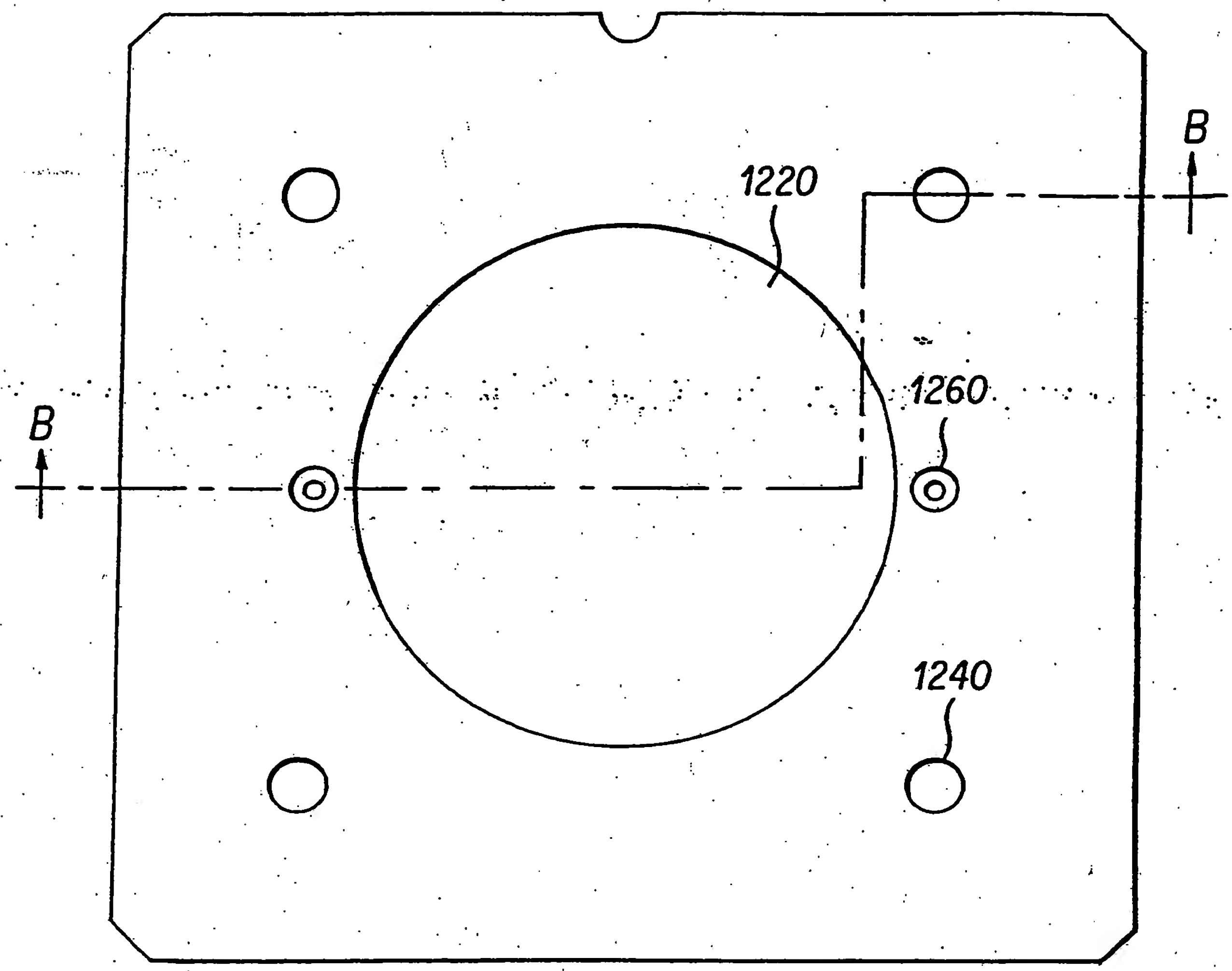

FIG. 4a

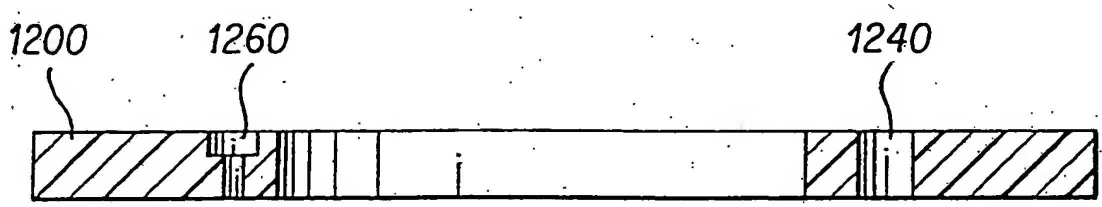

FIG. 4b

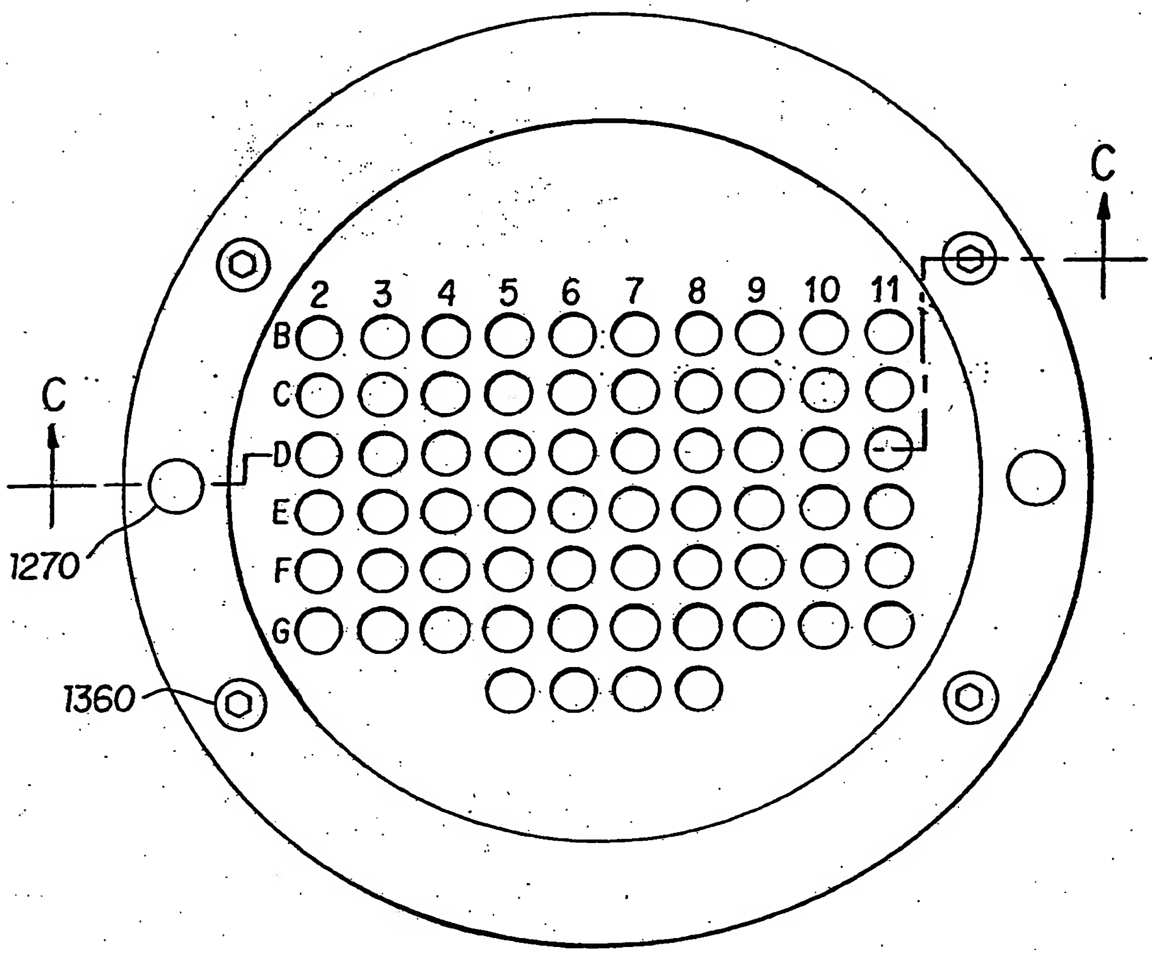
C
C
2
3
4
5
6
7
8
9
10
11
B
C
D
E
F
G
1270
1360

FIG. 5a

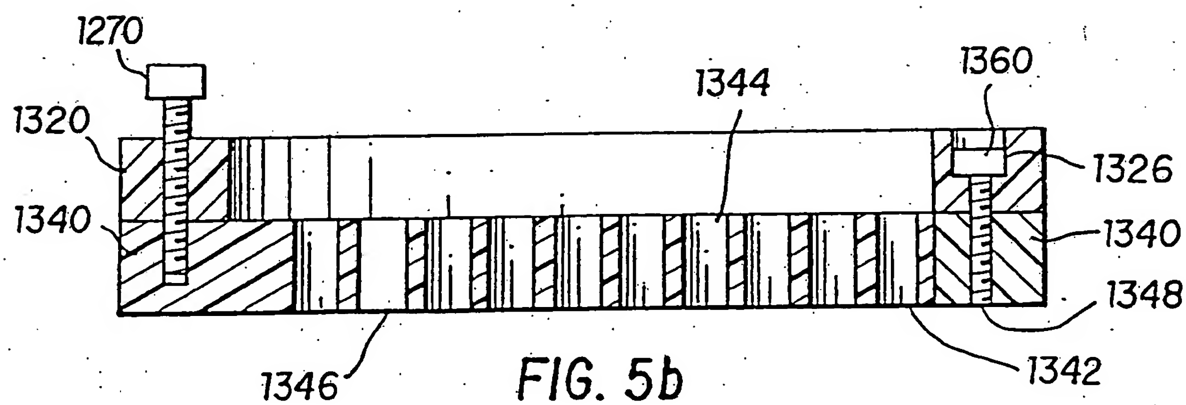
1270
1320
1344
1360
1326
1340
1340
1348
1342
1346

FIG. 5b

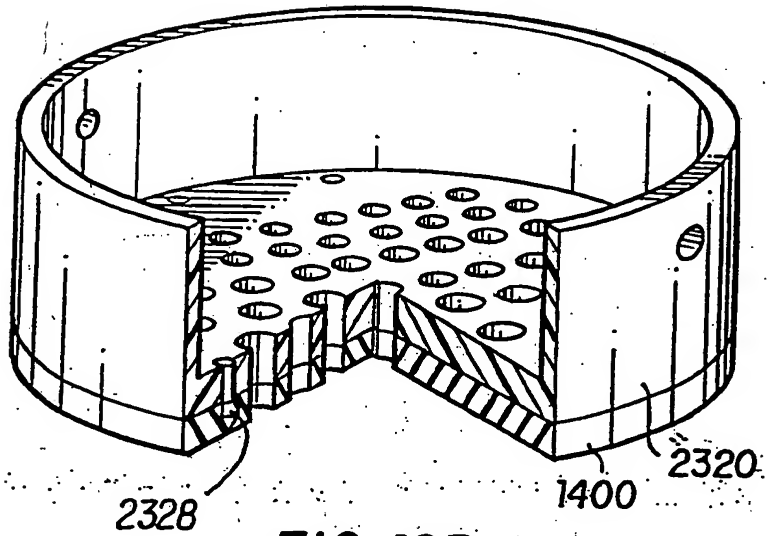

FIG. 12B

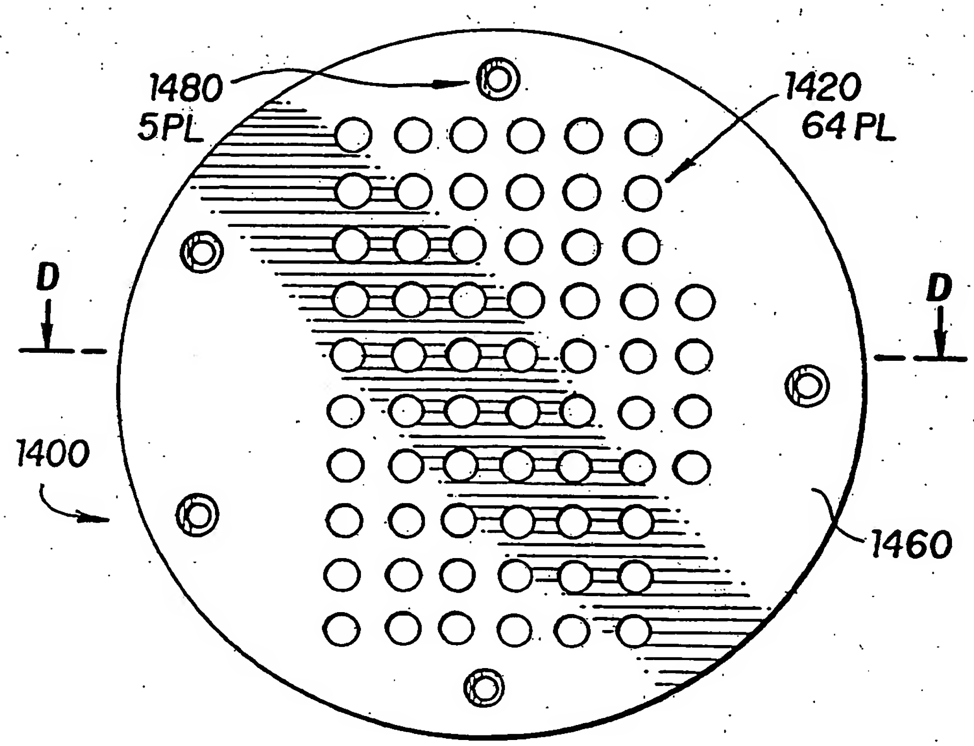

FIG. 6A

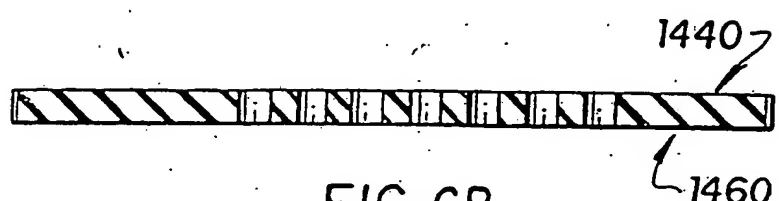

FIG. 6B

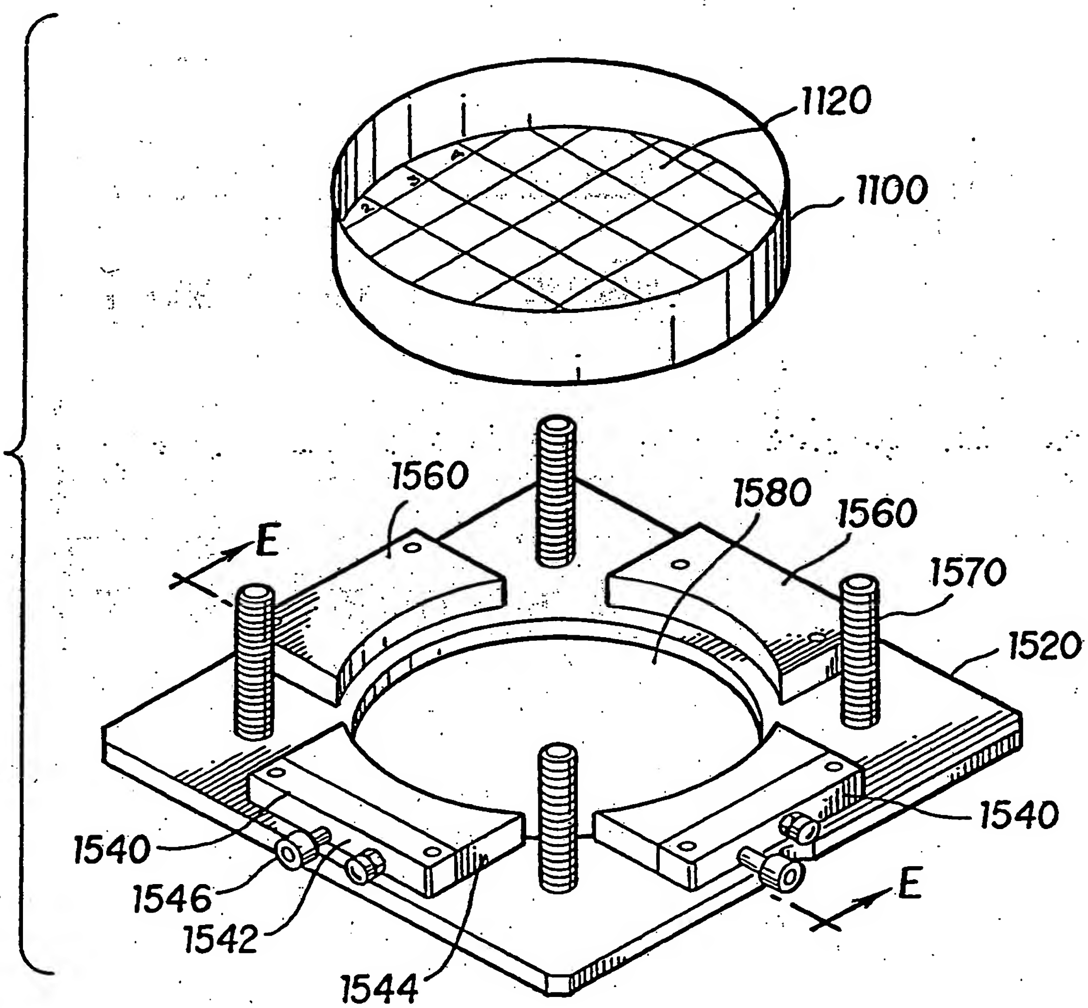
1120
1100
1560
1580
1560
E
1570
1520
1540
1540
1546
1542
1544
E

FIG. 7a

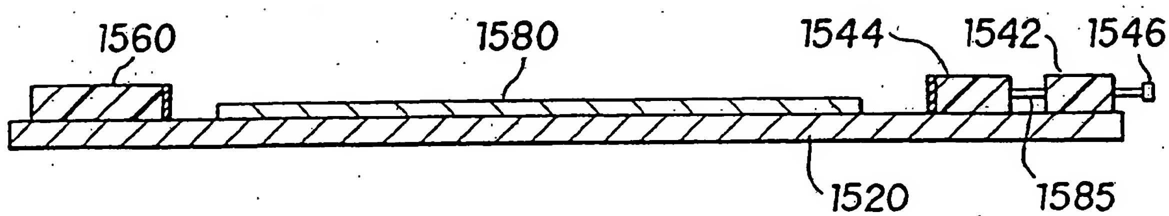
1560
1580
1544
1542
1546
1520
1585

FIG. 7b

PLANT CELLS IN 150mm DISH

WASH DISH AND COOL TO 4°C

ASSEMBLE CHAMBER ON DISH

REMOVE BUFFER, ADD SUPERNATANTS TO EACH WELL, INCUBATE

REMOVE SUPERNATANTS, WASH BY INVERTING CHAMBER

DISASSEMBLE CHAMBER

ADD ANTI-GLOBULIN-RHODAMINE SECOND STEP, INCUBATE

WASH, THEN FIX

WASH, THEN ADD GLYCEROL

ADD TEMPLATE PATTERN TO BOTTOM OF DISH

EXAMINE USING UPRIGHT MICROSCOPE

FIG. 8

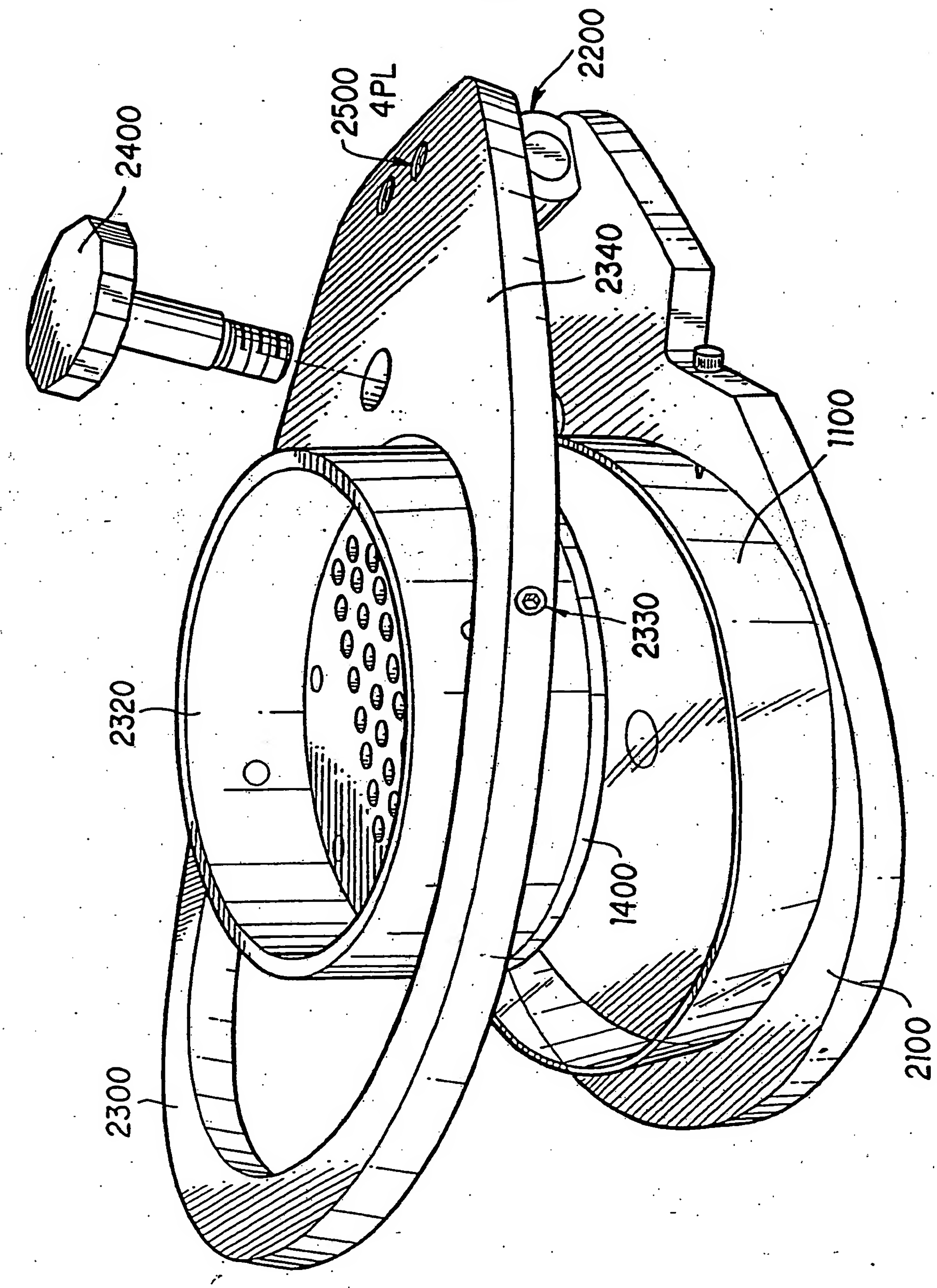

FIG. 9

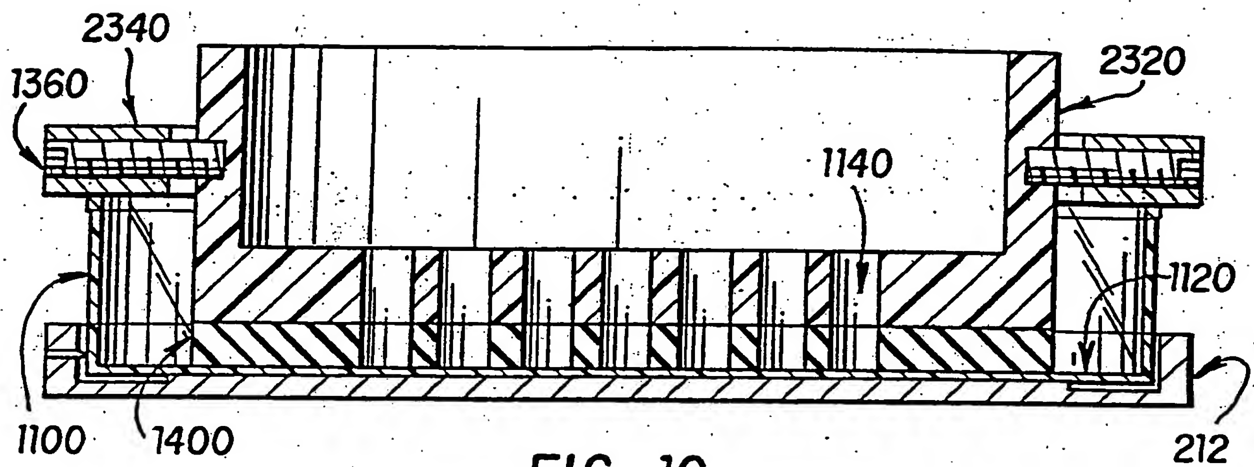

FIG. 10

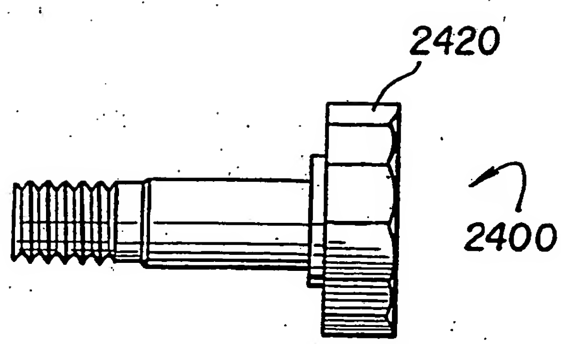

FIG. 14

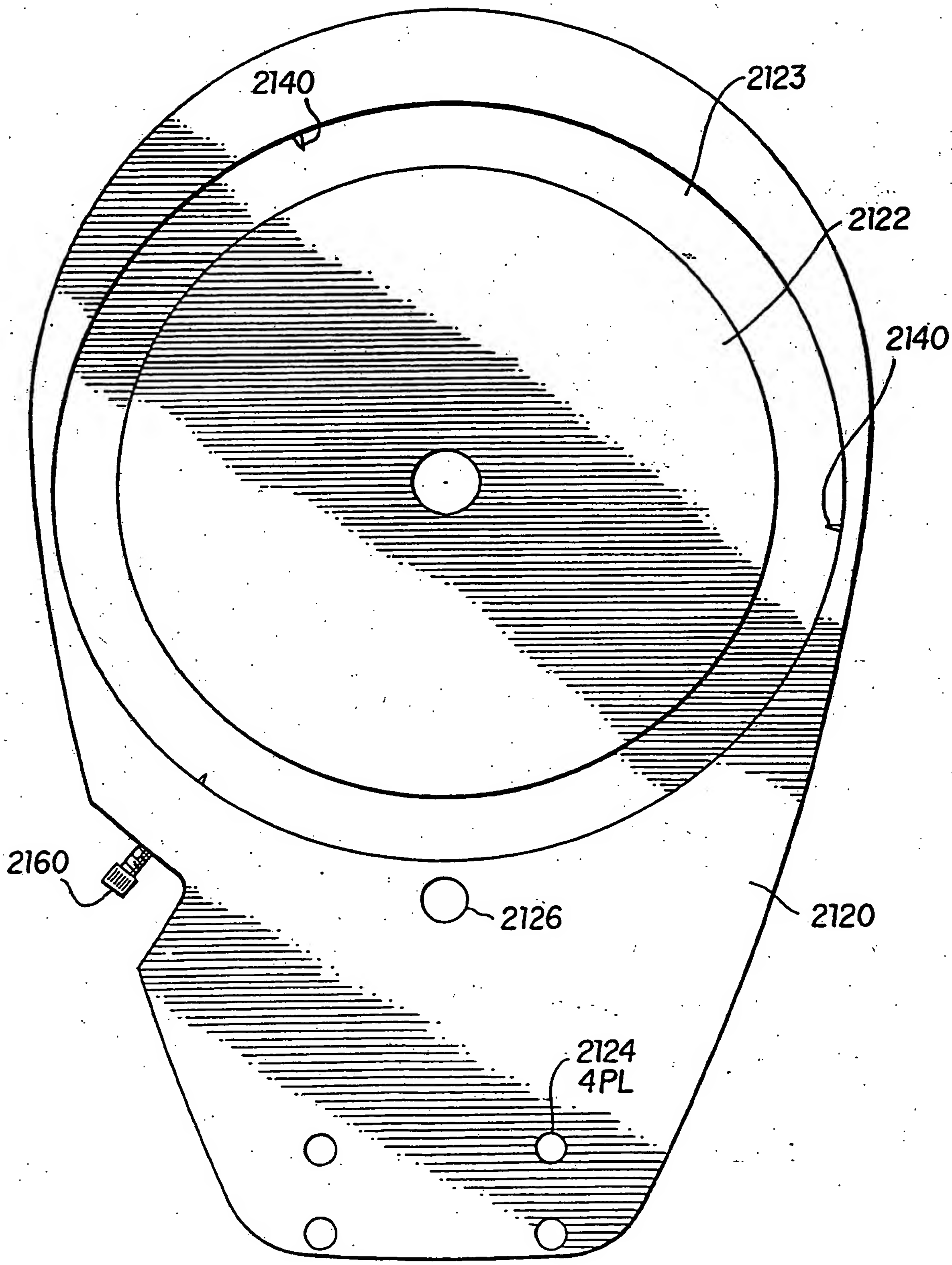

FIG. 11

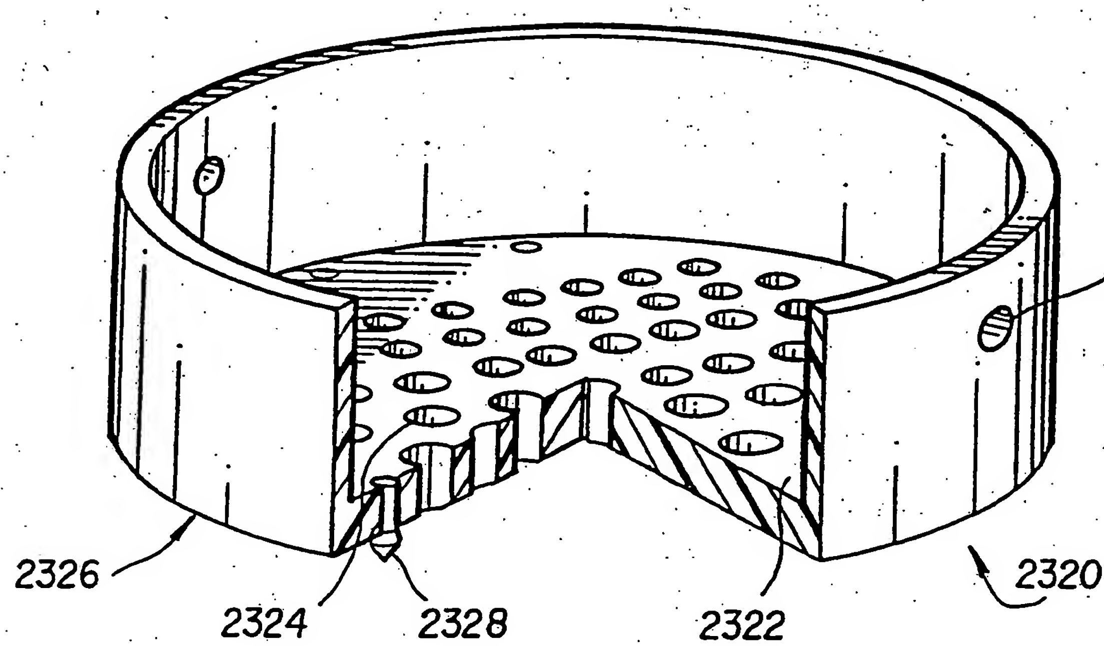

FIG. 12A

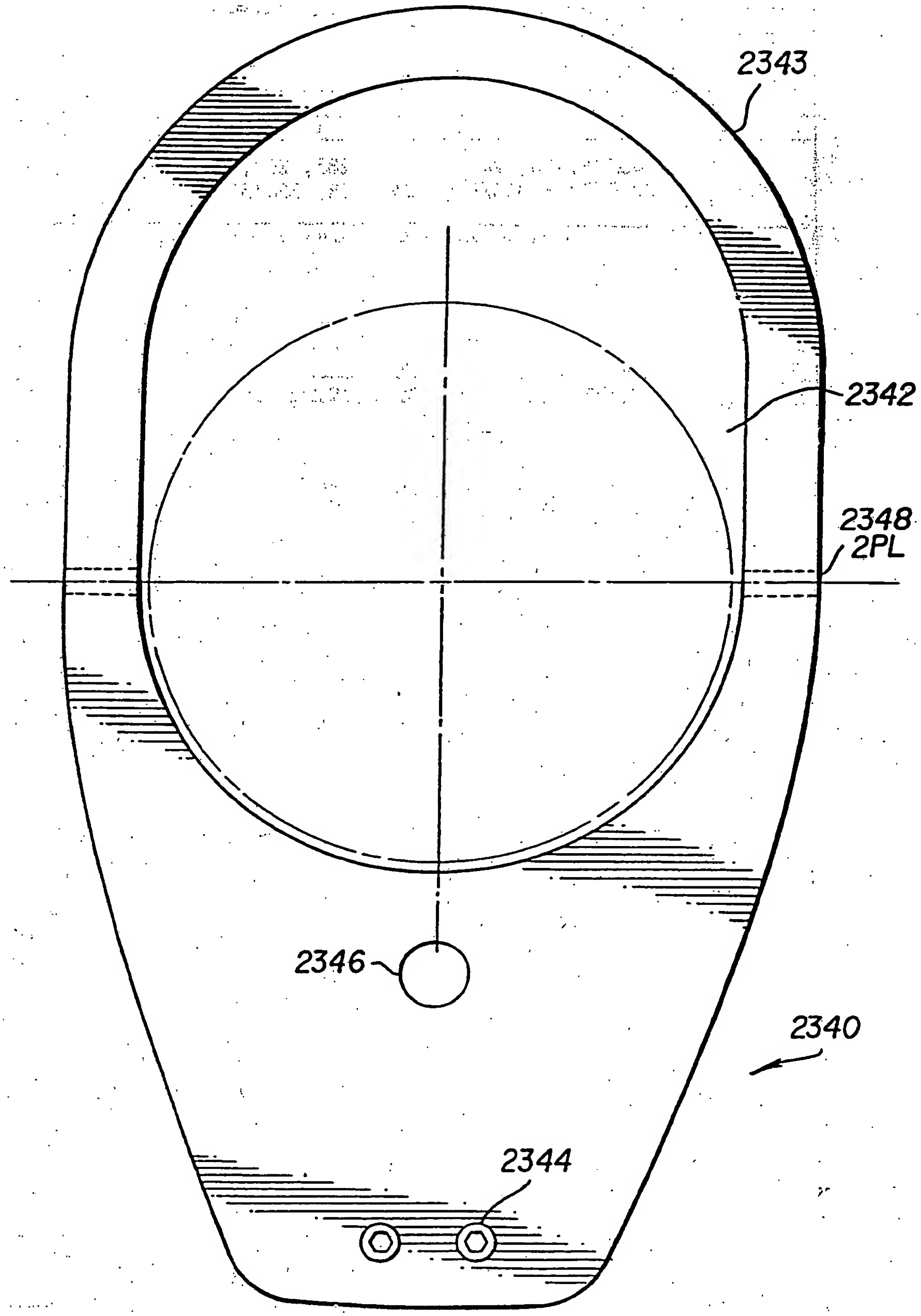

FIG. 13

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US90/07545

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C12Q 1/24; C12M 3/00

U.S. CL.: 435/30, 284

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/4, 30, 240.23, 284,285, 297, 298, 300, 301; 422/99,102; 250/576; 356/244, 246, 38; 350/534, 536

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X Y	US, A, 4,039,247 (LAWMAN ET AL.) 02 August 1977, see entire document.	1-4,7,8 5,6
X Y	Journal of Immunological Methods, Volume 125, No. 1-2, issued 20 December 1989, LR. Berghman et al., "Development of a Novel Screening Device Permitting Immunocytochemical Screening of Numerous Culture Supernatants During Hybridoma Production", pages 225-233, see entire document.	1-4,7,8 5,6
A	US, A, 3,540,985 (Gross) 17 November 1970, see entire document.	1-8
A	US, A, 3,736,042 (MARKOVITS ET AL.) 29 May 1973, see entire document.	1-8
A,P	US, A, 4,912,057 (GUIRGUIS ET AL.) 27 March 1990, see entire document.	1-8

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 March 1991	04 APR 1991

International Searching Authority	Signature of Authorized Officer
ISA/US	William H. Beisner ebw

Form PCT/ISA/210 (second sheet) (rev.11-87)